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Original antigenic sin shapes the immunological repertoire evoked by HCMV gB-MF59 vaccine in seropositive recipients

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Summary:

Vaccination of HCMV infected individuals with the glycoprotein B vaccine boosts pre-existing immune responses against gB but fails to induce new responses against novel linear epitopes within gB in seropositive individuals.

Abstract:

A cytomegalovirus (CMV) vaccine is urgently needed to protect against primary infection and enhance existing immunity in CMV infected individuals (CMV+). Using sera from CMV+ gB/MF59 vaccine recipients prior to transplant we investigated the composition of the immune response. Vaccination boosted pre-existing humoral responses in our CMV+ cohort but did not promote *de-novo* responses against novel linear epitopes. This suggests that prior natural infection has a profound effect on shaping the antibody repertoire and subsequent response to vaccination ('original antigenic sin'). Thus vaccination of CMV+ may require strategies of epitope presentation distinct from those intended to prevent primary infection.

Keywords: cytomegalovirus, vaccination, antibody responses, original antigenic sin

Main text:

Background:

Human Cytomegalovirus (HCMV) infection is common, with seroprevalence ranging from 60 to 100% (1). HCMV can promote substantial mortality and morbidity in immunocompromised individuals, including solid organ transplant (SOT) recipients (2). In these patients, CMV end-organ disease results from primary infection, reinfection or reactivation (3). The most successful vaccine studied to date is recombinant glycoprotein-B (gB) with MF59 adjuvant, which demonstrated partial efficacy in reducing viraemia after SOT and similar efficacy in preventing primary infection in women and adolescents (4, 5). While the mechanism of protection is not fully understood we have previously reported that higher levels of total anti-gB IgG antibody correlated with a shorter duration of post transplantation viraemia (6).

In CMV+ individuals the vaccine clearly boosted pre-existing antibody responses (7). Furthermore, detailed analyses of humoral responses against well-defined antigenic domains (AD1, AD2, AD4, and AD5) in seropositive individuals revealed that only anti-AD2 antibody responses correlated with protection from post-transplantation viremia. Importantly, vaccination only boosted AD2 responses in the 50% of CMV+ individuals with a pre-existing response and did not induce a new AD2 response in those who lacked AD2 antibodies following natural infection. Although there was no evidence that the potent responses towards AD1, AD4 and AD5 impaired protection from AD2, it is possible that a large proportion of the antibodies elicited by natural infection (and thus boosted by vaccination) are non-protective (7, 8). We hypothesized that highly immunogenic domains that induce non-protective responses might facilitate CMV replication by diverting immune system resources away from domains that might induce more protective responses (7, 9, 10). To begin addressing this interesting question we used peptide array technology for scanning antibody responses to linear gB epitopes across all protein domains in six CMV+ SOT recipients.

Methods:

Patient population

The sub-population from whom samples have been evaluated and described in this work are the cohort of solid organ transplant patients who were enrolled in the phase-2 randomised and double-blinded placebo controlled cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant trial. This trial was registered with ClinicalTrials.gov, NCT00299260 (6). The vaccine or placebo was given in three doses: at Day 0 (baseline), 1 month and 6 months later. Following vaccination, the blood samples from patients were obtained consecutively. The first five blood samples were collected before transplantation in order to measure antibodies (qualitatively and quantitatively) at baseline, and after 1, 2, 6 and 7 months. The patients who subsequently underwent transplantation were followed up for 90 days during which serial blood samples were obtained around days 0, 7, 35, 63, 90 post-transplant. The level of viral DNA was also tested by measuring CMV DNA by real-time quantitative PCR (RTqPCR) (6). Exclusion criteria included: pregnancy (a negative pregnancy test was required before each vaccine dose); receipt of blood products (except albumin) in the previous 3

months, and simultaneous multi-organ transplantation (6). The study was approved by the Research Ethics Committee and all patients gave written informed consent (6).

gB peptide microarray

To identify linear gB epitope binding, 15-mer peptides covering the entire gB open reading frame (Towne strain), and overlapping with neighbouring peptides by 10 residues (total of 188 peptides) were synthesized and printed to a PepStar multiwell array (JPT Peptide) in triplicate. Microarray binding was performed manually using individual slides immobilized in the ArraySlide 24-4 chamber (JPT Peptide). First, arrays were incubated for 1 hour with sera diluted 1:200 in blocking buffer (Superblock T20 (TBS), ThermoFisher Scientific) followed by a 1 hour incubation with anti-human IgG conjugated to AF647 (Jackson ImmunoResearch) diluted in blocking buffer (0.1 µg/mL). Following each incubation step, arrays were washed 5x in wash buffer (1x TBS buffer + 0.1% Tween) using an automated plate washer (Wellwash Versa). Array was then dried by centrifugation and scanned at a wavelength of 635 nm using an Axon Genepix 4300 SL50 scanner (Molecular Devices) at a PMT setting of 650 and 100% laser power. Images were analysed using Genepix Pro 7 software (Molecular Devices). Images were reviewed manually for accurate automated peptide identification. For each spot, mean signal intensity was extracted. For each peptide, the MMC2 values were calculated (the mean values of all three instances on the microarray, except when the coefficient of variation (CV) was larger than 0.5. In this case the mean of the two closest values (MC2) was assigned to MMC2). Data analysis and graphical presentations were made using the software R.

Results:

To characterise the antibody profile against linear epitopes of gB the sera of six CMV+ gB/MF59 vaccine recipients were analysed pre and post-vaccination (Fig.1; Fig.S.1; Fig.S.3).

This allowed the identification of epitopes recognised during natural infection as well those induced or boosted by vaccine. Responses to several previously reported epitopes were observed including some located in the Cytosolic Terminal Domain (CTD). Studies of the serological responses to this region are limited with two studies from the early 90s showing high serum reactivity to this region, subsequently called "AD3" (11, 12). It was speculated that, due to its location on the intraluminal, cytosolic part of gB, antibodies against this region will be most likely non-neutralising and non-protective. Perhaps this assumption explains why AD3 has not been given sufficient attention as a potential antibody target in the past. However, Nelson et al (13) recently analysed sera from a cohort of CMV- post-partum women vaccinated with gB/MF59 and subsequently found that 76% of the vaccine-induced linear IgG response recognized CTD/AD3.

Our work with CMV+ sera shows that this also happens after natural infection demonstrating that an overwhelming majority of all anti-gB antibodies against linear epitopes were specific for this region (Fig.1.B). Interestingly, vaccination boosted pre-existing anti-CTD responses to an extremely high level in three patients, dwarfing the responses observed to other ADs (Fig.1.C and Fig. S1). The same three patients experienced post-transplantation CMV viraemia. In direct contrast the remaining three patients who had not developed these antibody responses subsequently following vaccination and had no evidence of post-transplantation viraemia (Fig.1.D).

Next, we sought to investigate how such potent response towards CTD in these three individuals correlated with production of antibodies towards other regions (Fig.2.) Interestingly we could see that high level of antibodies to AD2 and CTD are mutually exclusive. This could potentially suggest that high level of anti-CTD antibodies could hinder generation of anti-AD2 responses, a response that we and others have previously demonstrated to be correlated with protection (Fig 2B) (8). Although such a small number of individuals preclude definite conclusions, our results argue that future studies should further investigate this highly immunogenic, cytosolic region of gB and its relationship with other antigenic domains of gB.

Discussion:

Based on this study of linear epitopes, our data suggest that vaccinating CMV+ individuals with the gB/MF59 vaccine predominantly boosts pre-existing antibody responses rather than inducing *de novo* responses. It is intriguing that while CTD is highly immunogenic, responses to this region appear to inversely correlate with protection from viraemia. One hypothesis is that inducing a humoral response against CTD CMV diverts the immune response away from targets more likely to induce protective antibody responses i.e. AD2. A competition model is not unique in HCMV whereby it is argued AD1 responses may interfere with protective AD2 responses - although in our patient cohort we did not observe a correlation between AD1 responses and the presence/absence of post-transplantation viremia (8). Additionally, we cannot rule out the reason for differences in protection are related to differences in the responses to other important targets for neutralisation (e.g. gH/gL complexes).

An important implication of this study is that vaccination of CMV+ individuals with gB/MF59 might simply boost the pre-existing antibody responses and, furthermore, in some individuals these might be non-protective. This concept is consistent with the paradigm of "original antigenic sin", which describes the tendency of the immune system to preferentially utilize immunological memory originating from a previous antigen encounter. Thus, the 'original antigenic sin' might be responsible for shaping the repertoire of immunological responses evoked by either vaccination or secondary exposure to different versions of the same pathogen (e.g. a different strain, or a recombinant protein subunit). As a result, pre-existing responses are boosted instead of vaccination promoting the development of novel protective responses that may occur in response to a newly encountered antigen. This phenomenon is well established with studies of Influenza, Dengue, and HIV, and considered to be a substantial obstacle to successful vaccine development (14). In this report we show, for the first time, that this immunological phenomenon could also hamper the success of the HCMV gB/MF59 vaccine in certain individuals. This becomes prescient if we consider that a successful vaccine against this highly prevalent pathogen should not only protect against primary infection but also re-infection with a different strain of the virus as well as re-activation of latent infection (1, 15).

We believe that this observation – albeit based on small numbers – illustrates the complexity of developing a universal vaccine strategy against a persistent viral infection highly prevalent in the population. It also supports the hypothesis that deletion of specific regions of gB, or alternative strategies to present gB, may be important – particularly in individuals with prior exposure to HCMV.

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Footnotes:

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Conflict of Interest:

Funding sources (Rosetrees Trust, Stoneygate Trust, Royal Free charity and MRC) had no role in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or in the decision to submit to publication. F.K. and P.H. are employees of JPT. All authors have submitted ICMJE forms for disclosure of potential Conflicts of Interest.

Ethics statement:

The study was approved by the Research Ethics Committee and all patients whose samples were investigated here gave written informed consent (6).

Meeting(s) where the information has previously been presented:

n/a

Figure 1. Responses against cytosolic terminal domain (CTD, AD3) in seropositive individuals are dominant and non-protective.

A) Linear structure of defined glycoprotein B antigenic domains. The entire open reading frame (ORFs) of HCMV gB are shown. The four distinct regions of the gB structure are indicated by black bars at the base of the figure, including the ectodomain, membrane proximal domain (MPD), transmembrane domain (TM), and the cytoplasmic domain. Major antigenic regions indicated include AD1 (orange), AD2 site 1 (red), AD2 site 2 (yellow), AD3 (purple), AD4 (Domain II) (green), and AD5 (Domain I) (blue). Numbers indicate approximate amino acid residues dividing each region of interest. Diagram was adapted from Burke et al., Plos Pathogens, 2015 and Nelson et al., PNAS, 2018. B-C). The highest values of antibody responses against these five major antigenic domains prior to vaccination (B) and following vaccination (C) are shown for each naturally seropositive SOT patient from R+ group. D) The highest value of IgG antibody responses against immunodominant AD3 region are shown for each patient prior to vaccination and post-vaccination. Median values of antibody responses are depicted by horizontal lines. Patients were further stratified for viraemia post-transplant (>200 viral genomes/ml of whole blood).

Fig.2. High level of antibodies to AD2 and CTD (AD3) are mutually exclusive.

A-D) The highest IgG response against AD1 (A), AD2 (B), AD4 (C) and AD5 (D) was plotted alongside the respective responses against cytoplasmic terminal domain (CTD/AD3); (n=6).

Figure 1

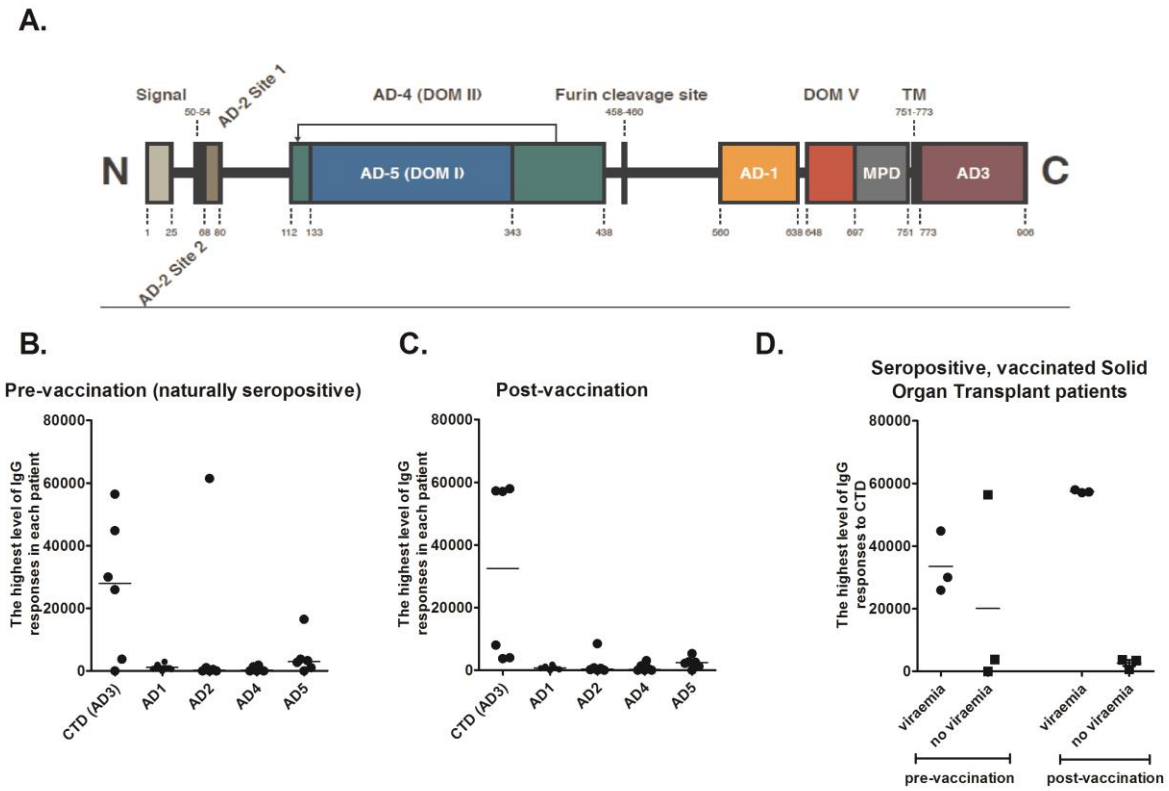

Figure 2